REMARKS

Applicant respectfully requests entry of the remarks submitted herein. Claims 1-19 are currently pending. Reconsideration of the pending application is respectfully requested.

Interview Summary

Applicant's Representative wishes to thank Examiner Audet for the courtesies extended to her during the personal interview on October 26, 2005. During the interview issues relating to the art cited by the examiner were discussed.

Claims Rejections under 35 U.S.C. §103(a)

The examiner indicated that claims 1-27 are rejected under 35 U.S.C. 103(a) as being unpatenable over Schwartz et al. (2003/0175762). Applicant presumes that examiner meant that claims 1-19 are rejected as there are only 19 claims currently pending in the present application. Applicant also presumes that the examiner meant that the claims are rejected over Schwartz et al. (2003/0232352), as publication number 2003/0175762 is to Nunez et al. Applicant further presumes that the examiner meant that the claims are unpatentable over Schwartz et al., in view of Khan et al. (2003/0119720), Calandra et al. (2002/0192217) and Nunez et al. (2003/0175762), since these references are also discussed in the Office Action.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. MPEP Section 706.02(j).

The examiner has not presented a *prima facie* case of obviousness because all the elements of the claimed invention have not been taught by the cited references, and because there is no suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference

teachings. Further, the inventor discovered an unexpected result regarding the interaction of CD14, endotoxin and MD-2, namely that endotoxin forms a complex with MD-2 in the presence of CD14.

Independent claim 1 recites a purified complex comprising endotoxin bound to MD-2. The inventor discovered that the complex of endotoxin bound to MD-2 is generated by treatment of endotoxin-sCD14 with MD-2 (see, *e.g.,* specification at page 18, line 1 to page 19, line 22; page 28, lines 21-25; and page 32, lines 25-27). At various places in the literature, endotoxin is also be called lipopolysaccharide (LPS) or lipooligosaccharide (LOS). As indicated in the drawing attached to the end of this Reply, aggregated endotoxin (E_{agg}) forms a complex with lipopolysaccharide binding protein (LBP), which is designated E^*_{agg}:$(LBP)_n$. sCD14 then extracts a single endotoxin molecule ($E_{6\text{-}FA}$) to form an $E_{6\text{-}FA}$:sCD14 complex. Next, the $E_{6\text{-}FA}$:sCD14 complex comes into contact with sMD-2 to form the $E_{6\text{-}FA}$:sMD-2 complex. When this $E_{6\text{-}FA}$:sMD-2 complex binds to the TLR4 receptor, pro-inflammatory responses are initiated. That an endotoxin molecule first binds to CD14 in order to be properly presented to MD-2, and that E:MD-2 formed prior to the MD-2 or endotoxin coming into contact with TLR4 can potently activate TLR4 were unexpected results. See, *e.g.*, page 33, lines 10-11 and Table 1 of the specification. Prior to the inventor's work, the role of CD14 and MD-2 was not known, as is evident from the references cited by the examiner (and discussed below).

Schwartz et al. disclose toll-like receptor-4 (TLR4) mutations, methods of assessing the susceptibility of an individual to atherosclerosis, and a method of treating an individual identified as being at increased risk (2003/0175762 at ¶0005). They disclose common TLR4 mutations that attenuate endotoxin signalling and diminish the host inflammatory response to inciting Gram negative pathogens that are associated with a decreased atherosclerosis risk, and that endotoxin plays a role in atherogenesis (2003/0175762 at ¶0010). They further disclose that the main ligand of TLR4 is LPS from common Gram negative bacteria including *C. pneumoniae* and *H. pylori*, which are the two pathogens most commonly implicated in human atherogenesis (2003/0175762 at ¶0010).

In the Schwartz et al. study to determine the ability of various TLR4 alleles to signal in response to LPS, THP-1 cells were transfected with various human TLR4 isoforms. They indicate that "the transfection mixes included human MD-2" (2003/0175762 at ¶0019). On the

next day, the cells were stimulated with LPS (*i.e.*, endotoxin) prior to lysis (2003/0175762 at ¶0019). It should be noted that the MD-2 to which they refer is not the MD-2 protein but cDNA for expression of MD-2 in THP-1 cells. LPS is added after transfection of cells. No MD-2 is added with the LPS, contrary to what is intimated by the examiner. Rather the added LPS (relatively high concentration of LPS is added) will see MD-2 that has been expressed by the cells along with TLR4, thus presenting MD-2 as a complex with TLR4 on the host cell membrane. This differs from the present invention, which is a purified complex of MD-2 with LPS. This experimental design of Schwartz et al. reflects the conventional wisdom at the time, which was that MD-2 functions in the context of its prior association with TLR4 on the host cell surface, and that TLR4 interacted with CD14 (2003/0175762 at ¶0004). They did not anticipate that MD-2 could react directly with LPS (if LPS is presented properly), independent of TLR4, and then subsequently a MD-2: E complex reacts with and activates TLR4.

Nowhere in the Schwartz et al. reference do they teach a purified complex comprising endotoxin bound to MD-2, as recited by the present claims. Further, Schwartz et al. do not teach that CD14 needs to bind to the endotoxin before the endotoxin is presented to MD-2, otherwise the endotoxin will not bind efficiently to MD-2 nor form a bioactive monomeric endotoxin:MD-2 complex. In fact, the belief of Schwartz et al. that LPS is the main ligand of TLR4 is contrary to the current claims that demonstrate that a monomeric complex of LPS (endotoxin) with MD-2, not LPS alone, is the ligand for TLR4-dependent cell activation. The observations and claims of Schwartz et al. predict neither the formation nor the stability of a monomeric complex nor the ability of such a complex to directly activate TLR4 as shown by the current claims. Therefore, the claims are not obvious over Schwartz et al.

Khan et al. do not remedy the deficiencies of Schwartz et al. Khan et al. disclose a method of treating disease, particularly anthrax (2003/0119720 at ¶0002). They disclose that the recognition of Pathogen Associated Molecular Patterns (PAMPS) provides for differential recognition of pathogens by TLRs. For example, TLR2 is generally activated in response to BLPs, PGNs of gram-positive bacteria, LAM of mycobacteria, and mannans of yeasts, whereas TLR4 is often activated by LPS of gram-negative bacteria and LTA of gram-positive bacteria; also a secreted small molecule MD-2 can account for TLR4 signaling (2003/0119720 at ¶0074). This is the only passage in Khan et al. that even mentions MD-2. Nowhere in Khan et al. do they

teach a purified complex comprising endotoxin bound to MD-2, as recited by the present claims. The Khan et al. reference in fact, teaches away from the present invention, as it implies that LPS can on its own can induce TLR4 activation (2003/0119720 at ¶0074). Therefore, the claims are not obvious over Khan et al.

Calandra et al. do not remedy the deficiencies of Schwartz et al. and Khan et al. Calandra et al. disclose methods for inhibiting the release and/or biological activity of migration inhibitory factor (MIF), and the use of such methods for the treatment of various conditions involving a mediator-induced diseases or pathology (2002/0192217 at ¶0002). They analyzed the expression of membrane-bound CD14 and TLR4-MD-2 complex (2002/0192217 at ¶0221). They also investigated the molecular mechanism by which MIF regulates response of macrophages to LPS and gram-negative bacteria. In particular they measured the mRNA levels of MD-2, and found that they were similar in control and antisense MIF macrophages (2002/0192217 at ¶0225). Calandra et al. teach away from the present invention, as they state that recognition of LPS requires the cooperative interplay between LBP, CD14 and TLR4. Calandra et al. state that LBP binds and transfers LPS-containing particles to a receptor complex composed of CD14, a ligand-binding GPI-anchored protein, and TLR4, the molecule that transduces the LPS signal (2002/0192217 at ¶ 0224). Calandra et al. makes no mention of the need for MD-2. Nowhere in Calandra et al. do they teach a purified complex comprising endotoxin bound to MD-2, as recited by the present claims. Therefore, the claims are not obvious over Calandra et al.

Nunez et al. do not remedy the deficiencies of Schwartz et al., Khan et al. and Calandra et al. Nunez et al. disclose that in humans, the TLR4/MD2/CD14 complex has been demonstrated to serve as a surface receptor for LPS (2003/0175762 at ¶0052). They further disclose that cell surface molecules such as MD2 and CD14 are required for TLR4-mediated LPS responses in cells, and that they co-transfected TLR4, CD14 and MD2 expression plasmids into HEK293T cells, which induced 8-fold activation of NF-κB (2003/0175762 at ¶0384). Nowhere in Nunez et al., however, do they teach a purified complex comprising endotoxin bound to MD-2, as recited by the present claims. Nor do Nunez et al. predict that cells containing only TLR4 (no CD14 or MD-2) could be potently activated by a soluble extracellular complex of endotoxin bound to MD-2 (no CD14). Therefore, the claims are not obvious over Nunez et al.

As discussed above, none of the cited references teach or suggest a *purified* complex comprising endotoxin bound to MD-2, as recited by the claims. Because none of the cited art references teach or suggest all the claim limitations, a *prima facie* case of obviousness has not been established. Further, no one predicted the unexpected result that cells containing only TLR4 (no CD14 or MD-2) could be potently activated by a purified complex of endotoxin bound to MD-2 (no CD14).

The examiner states that it would have been obvious to one of ordinary skill in the art at the time of the invention to administer LPS and MD2 in a bound form, rather than a singular form as discussed in Schwarz et al., because Khan et al., Calandra et al., and Nunez et al., all teach the advantageous synergistic effect of LPS and MD2 on TLR4 signaling, and because Schwartz et al. teach the singular administration of LPS and MD2 for the same effect. Thus, one of ordinary skill in the art would be motivated to administer LPS and MD2 in bound form (or singular form), in order to elicit the synergistic signaling effect desired in the TLR4 pathway.

Applicant respectfully disagrees that Khan et al., Calandra et al., and Nunez et al., all teach the advantageous synergistic effect of LPS and MD2 on TLR4 signaling. It is true that it was known in the art that LPS and MD2 have an effect on TLR signaling. It was not known, however, that the endotoxin:MD-2 complex could activate cells in a TLR4-dependent fashion without the inclusion of other host or bacterial factors (specification at page 18, lines 4-6). Further, it was not known until the present inventor made his discovery, that MD-2 had a direct role in endotoxin recognition and delivery of endotoxin to host cells containing TLR4, or that a prior association of MD-2 with TLR4 was not necessary prior to introduction of endotoxin, as was previously presumed (specification at page 18, lines 19-22). It was also not known until the present inventor made his discovery that a monomeric complex of endotoxin and MD-2 could act either as a TLR4 agonist or as a TLR4 antagonist, depending on the structural properties of the endotoxin and/or MD-2 molecules. Although the importance of LBP and CD14 was generally appreciated, it was not known until the present discovery that the extraction and transfer of endotoxin to CD14 was needed to permit efficient transfer of endotoxin to MD-2 and formation of bioactive monomeric E:MD-2 complex.

Accordingly, Applicant respectfully requests withdrawal of this 35 U.S.C. § 103(a) rejection of the claims.

Conclusion

Applicant respectfully requests a favorable examination of the merits of this patent application. The Examiner is invited to telephone Applicant's attorney at (952) 876-4091 to facilitate prosecution of this application. Please charge any fees deemed necessary to Deposit Account 50-3503.

Respectfully submitted,

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